FENCING CLAIMS AFTER FEFTININAFY AMENUMUNU

- 1. Method for detecting and/or purifying substances selected from proteins, biomplecules, complexes of proteins ar biomplecules, subunits thereof, cell components, cell organelles and cells comprising the steps;
- or more heterologous nucleic acids encoding one or more beterologous nucleic acids encoding one or more polypeptides and/or one or more subunits of a ciomolegule complex, the polypeptides or subunits being fused to at least two different affinity tags, one of which consists of one or more IgG binding domains of Staphylococcus protein A,
- b, maintaining the expression environment under conditions that facilitate expression of the one or more polypeptides or subunits in a native form as fusion proteins with the affinity tags,
- polypeptides or subunits by a combination of at least two different affinity purification steps each comprising binding the one or more polypeptides or subunits via one affinity tag to a support material capable of selectively binding one of the affinity tags and separating the one or more polypeptides or subunits from the support material after substances not boind to the support material after substances not
- 2. Method for detecting and/or purifying biomolecule and/or protein complexes, comprising the steps:
- providing an expression environment containing one or more heterologous musleur acids encoding at least two subunits vi a providence sorplex, each point fusually a contained to the Ceast one of different africatly tage, the or which substate is the or more log bunding domains of staphyl course protein A.

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- assistions that facilitate expression if the she is made subunits in a native form as fusion probeins with the attinity tags, and under conditions that allow the formation of a complex between the one or more subunits and possibly other components capable of complexing with the one or more subunits,
- condetecting and/or purifying the complex by a sumbination of at least two different affinity purification steps each comprising binding the one or more subunits via the affinity tag to a support material capable of selectively binding one of the affinity tags and separating the complex from the support material after substances not bound to the support material have been removed.
- 3. Method according to claim 1 or 2, wherein between the one or more polypeptides or subunits and one or more of the affinity tags a specific proteclytic cleavage site is gresent in the fusion protein which facilitates the removal of the or more of the affinity tags.
- 4. Method according to claim 3, wherein the specific proteclytic cleavage site is an enzymatic cleavage site.
- 5. Method according to claim 4, wherein the specific protectytic cleavage site is the cleavage site for TEV protease NIA.
- s. Method acturaling to black to the protection of the supplier of the protection of the supplier of the

Attarner Dat. No. 7 400 . Mering acomiding to place 6, wherein the affinity partitions a si step of respirates: (i) binding the one or more polyperblidge or subunits via the one or more Ig3 binding domains of Staphylinocous to a support material dapable of specifically binding the latter, removing substances not bound to the support material and separating the one or more polypeptides or subunits from the support material by cleaving off the IgS binding domains via the specific proteclytic cleavage site, and it binding the polypeptides or subunit via another affintly tag to a second support material capable of specifically binding the latter, removing substances not bound to the support material and separating the polypeptide or subunit from the support material. 8. Method according to claim 7, wherein step (ii) is carried out before step (i'. F. Method according to claim 3, wherein the fusion protein contains a second specific proteclytic cleavage site for the removal of one or more of the other affinity tags. 1). Method according to claim 1 or 2, wherein one if the

affinity tags consists of at least one dalmodulin hinding

agent is used to separate the one or more polypeptides or

on subponit of a postein complex forest to at least two

subunits from the support material.

11. Method according to claim 40, wherein a chemical

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peptide.

infrement affinity tags, wherein the of the allienty tags consists of at least one lighternains account to Staphyloc. The protein A.

- 13. Fusion protein according to claim 12, wherein it additionally contains a specific proteolytic cleavage site.
- 14. A nucleic acid toding for a fusion protein, the fusion protein comprising at least one polypeptide or subunit of a protein pomplex fused to at least two different aftimity tags, wherein one of the affinity tags consists of at least one IgS binding domain of Staphylococcus protein A.
- of sequences facilitating the expression of a fusion protein, the fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein one of the affinity tags consists of at least one IgG kinding domain of Staphyldcocous protein A.
- 16. Vector comprising heterologous nucleic acid sequences in form of one or more cassettes each comprising at least two different affinity tags one consisting of the interesting distance of staphylogogous aureus protein. A, and at least one polynucleotide linker for the insertion of further nucleic acids.
- 17. Vector comprising heterologous nucleic acid sequences in form of two or more cassettes each comprising at least one of different affinity tags the ponsisting of one or more last linding dimarns of scaphylococcus sure a platfell A, and at least one polymorlecture linker for the insential of

protein or a vector comprising a nucleur and builder the control of sequences facilitating the expression of a fusion protein, the fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein one of the affinity tags consists of at least one lgG binding domain of Staphylococcus protein A.

19. A reagent kit comprising:

a nucleic acid coding for a fusion protein or a vector for the expression of a fusion protein, wherein the fusion protein comprising at least one polypeptide or subunit of a protein complex fused to at least two different affinity tags, wherein one of the affinity tags consists of at least one IgG binding domain of Staphylococcus protein A; and

support materials each capable of binding at least treaffinity tag.

- 20. Reagent kit according to claim 19 additionally numerising at least one chemical agent for separating one if the affinity mags from its support material and/or a specific chemical protection agent and/or specific protease capable of pleasing the fusion protein.
- 23. A nucleic acid according to claim 14 or 18 wherein the fueron profess further comprises a specific protectivity of the same state.
 - 14. The reason Rit of Claim to wherein the vector

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includes a nucleic acid under the goutest of sequebots tactification the expression of tastic proteins.

- comprising heterologous numbers acid sequences in form of one or more cassettes each comprising at least two different affinity tags one consisting of one or more Ig3 cinding domains of Staphylococcus aureus protein A, and at least one polynucleotide linker for the insertion of further nucleic acids.
- 26. The reagent kit of claim 19 wherein the vector comprises heterologous heterologous nucleic acid sequences in form of two or more cassettes each comprising at least one of different affinity tags one consisting of one or more IgG binding domains of Staphylococcus aureus protein A, and at least one polynucleotide linker for the further insertion of further nucleic acids.
- 27. A method for detection and or purification of substances capable of complexing with fusion proteins, the method comprising contacting the fusion proteins with a sample and detecting and/or purifying substances capable of complexing with the fusion protein.
- 28. A method for detection and/or purification of cells and/or cell organelles expressing a fusion protein on their surface, the method comprising contacting the cells and/or cell organelles expressing a fusion protein on their surface with a substance papable if binding with the fusion protein, and detecting and or purifying the Obil and or well from the expressing the fusion protein.